FORM PTO-1390 (REV 10-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371	33339/235735
	U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5) To be assigned 09/868399

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FR99/03311	December 29, 1999	December 31, 1998

TITLE OF INVENTION

DETECTING AND MONITORING OF HIV INFECTIONS

APPLICANT(S) FOR DO/EO/US

Denis Tranchand-Bunel; Claude Auriault

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☒ This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)).

4. ☒ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).

5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   - a. ☒ is attached hereto (required only if not communicated by the International Bureau).
   - b. ☒ has been communicated by the International Bureau.
   - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).

6. ☒ A English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).

7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   - b. ☐ have been communicated by the International Bureau.
   - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   - d. ☒ have not been made and will not be made.

8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. To 16. Below concern other document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☒ Other items or information: Statement in Support of filing a Sequence Listing Under 37 CFR 1.821(c) and (e); Sequence Listing; Computer redeable disk

09868399 .071201

JC18 Rec'd PCT/PTO 18 JUN 2001

U.S. APPLICATION NO. (if known, see 37 C.F.R. 1.5) To be assigned 09/868399	INTERNATIONAL APPLICATION NO. PCT/FR99/03311	ATTORNEY'S DOCKET NUMBER 33339/235735	
17. ☒ The following fees are submitted:		CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Neither international preliminary examination fee (37 CFR 1.482) nor International search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1,000.00** International preliminary examination fee (37 CRF 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00** International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search (37CFR 1.445(a)(2)) paid to USPTO **$710.00** International preliminary examination fee (37 CFR 1.482) paid to USPTO But all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00** International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$ 100.00**			
ENTER APPROPRIATE BASIC FEE AMOUNT =		**$ 860.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).		**$**	
CLAIMS	NUMBER FILED / NUMBER EXTRA / RATE		
Total Claims	13 -20 = 0 X $18.00	**$ 0.00**	
Independent Claims	1 - 3 = 0 X $80.00	**$ 0.00**	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)	+ $270.00	**$**	
TOTAL OF ABOVE CALCULATIONS =		**$ 860.00**	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by one-half.		**$**	
SUBTOTAL =		**$ 860.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).		**$**	
TOTAL NATIONAL FEE =		**$ 860.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +		**$**	
TOTAL FEES ENCLOSED =		**$ 860.00**	
		Amount to be Refunded	**$**
		Charged	**$**

a. ☒ A check in the amount of $ 860.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 16-0605 in the amount of $ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 16-0605.

Note: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

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Sarah B. Simmons

CLT01/4483376v1

Attorney's Docket No. 33339/235735

IN THE UNITED STATES DESIGNATED OFFICE (DO/US)

In re:	Tranchand-Bunel et al.	Attn.: DO/US
International Appl. No.:	PCT/FR99/03311	
International Filing Date:	December 29, 1999	
For:	DETECTING AND MONITORING OF HIV INFECTIONS	

June 18, 2001

STATEMENT IN SUPPORT OF FILING A SEQUENCE LISTING UNDER 37 CFR § 1.821(f)

Commissioner for Patents
Washington, DC 20231

Sir:

I hereby state that the content of the paper and computer readable copies of the Sequence Listing, submitted concurrently herewith in accordance with 37 CFR § 1.821(c) and (e), are the same.

Respectfully submitted,

Raymond O. Linker, Jr.
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"Express Mail" Mailing Label Number EL 822757964 US Date of Deposit: June 18, 2001 I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to Box PCT, Commissioner for Patents, Washington, DC 20231. Sarah B. Simmons	**CERTIFICATE OF MAILING** I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner For Patents, Washington, DC 20231, on June 18, 2001. _____

Attorney's Docket No. 33339/235735

IN THE UNITED STATES DESIGNATED OFFICE (DO/US)

In re:	Tranchand-Bunel et al.	Attn: DO/US
International Appl. No.:	PCT/FR99/03311	
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For:	DETECTING AND MONITORING OF HIV INFECTIONS	

June 18, 2001

Box PCT
Commissioner for Patents
Washington, DC 20231

PRELIMINARY AMENDMENT

Sir:

Please amend the above-identified application as follows:

In The Abstract:

Please add the following as page 15 of the application:

DETECTION AND MONITORING OF HIV INFECTIONS

ABSTRACT OF THE DISCLOSURE

The invention concerns a reagent for detecting and monitoring viral infections, caused by the human immunodeficiency virus (HIV) and its uses for detecting human immunodeficiency. The reagent comprises a mixture consisting of (1) an antigenic peptide coded by the HIV pol gene comprising 60 amino acids, preferably between 20 and 40 amino acids; and (2) a mixture (called a mixotope) of convergent combinatorial peptides, derived from said antigenic peptide.

In The Specification:

Please add the following as Line 3 on Page 1 of the application, between the title of the invention and the first paragraph:

FIELD OF THE INVENTION

Please add the following as Line 6 on Page 1 of the application, between the first and second paragraphs:

BACKGROUND OF THE INVENTION

Please add the following between numbered lines 22 and 23 on Page 3 of the application:

SUMMARY OF THE INVENTION

Please add the following between numbered lines 20 and 21 on Page 6 of the application:

BRIEF DESCRIPTION OF THE DRAWINGS

Please add the following between numbered lines 25 and 26 on Page 7 of the application:

ILLUSTRATIVE EXAMPLES

In The Claims:

1. (Amended) A reagent for detecting an infection caused by a human immunodeficiency virus, comprising a mixture consisting of (1) an antigenic peptide coded for by the *pol* gene of HIV-1 and comprising at most 60 amino acids, and (2) a mixture, called a mixotope, of convergent combinatorial peptides derived from said antigenic peptide.

2. (Amended) A reagent according to Claim 1, wherein said antigenic peptide corresponds to an epitope of the integrase coded for by the *pol* gene of HIV-1.

3. (Amended) A reagent according to Claim 2, wherein said antigenic peptide corresponds to the sequence KIQNFRVYYRDSRDPLWKGPAKLLWKGEGAVVIQDN (SEQ ID NO:1) (HIV-POL).

4. (Amended) A reagent according to Claim 1, wherein the mixotope corresponds to a degeneration of the whole of the selected antigenic peptide.

5. (Amended) A reagent according to Claim 1, wherein the antigenic peptide (1) and the mixotope (2) are attached to a solid support.

6. (Amended) A reagent according to Claim 5, wherein said antigenic peptide (1) and said mixotope (2) are attached to said support sequentially.

7. (Amended) A reagent according to Claim 1, wherein the ratio of antigenic peptide to mixotope in the mixture is between 1:10 and 1:100.

8. (Amended) An enzyme immunological method of diagnosing an HIV-1 infection, which employs a diagnostic reagent according to Claim 1.

9. (Amended) A method according to Claim 8, which comprises:

- bringing a serum to be analysed into contact with a reagent comprising a mixture consisting of (1) an antigenic peptide coded for by the *pol* gene of HIV-1 and comprising at most 60 amino acids, and (2) a mixture, called a mixotope, of convergent combinatorial peptides derived from said antigenic peptide;
- adding anti-human Ig antibodies coupled with an enzyme; and
- qualitatively and/or quantitatively disclosing the anti-integrase antibodies which may be present in the serum to be analysed by adding the enzyme substrate.

10. (Amended) A method according to Claim 8, which comprises:

- attaching to a support a reagent comprising a mixture consisting of (1) an antigenic peptide coded for by the *pol* gene of HIV-1 and comprising at most 60 amino acids, and (2) a mixture, called a mixotope, of convergent combinatorial peptides derived from said antigenic peptide;
- adding the serum to be analysed;
- detecting the attachment of the anti-integrase antibodies present in said serum by adding anti-human IgG antibodies coupled with an enzyme; and
- qualitatively and/or quantitatively disclosing said antibodies in a spectrophotometer by adding the enzyme substrate.

11. (Amended) A kit for diagnosing an HIV-1 infection, which comprises at least one reagent according to Claim 1.

12. (New) A reagent according to Claim 1, wherein said antigenic peptide comprises between 20 and 40 amino acids.

13. (New) A reagent according to Claim 5, wherein said solid support comprises a microtitre plate.

REMARKS

The above amendments are made to conform the specification and claims to United States practice. Please enter this amendment prior to calculation of the filing fee.

Respectfully submitted,

Raymond O. Linker, Jr.
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Sarah B. Simmons

CLT01/4483393v1

Version With Markings to Show Changes Made:

In the Claims:

1. (Amended) A reagent [Reagent] for detecting an infection caused by a human immunodeficiency virus, [characterized in that it comprises] comprising a mixture consisting of (1) an antigenic peptide coded for by the *pol* gene of HIV-1 and comprising at most 60 amino acids, [preferably between 20 and 40 amino acids,] and (2) a mixture, called a mixotope, of convergent combinatorial peptides derived from said antigenic peptide.

2. (Amended) A reagent [Reagent] according to Claim 1, [characterized in that] wherein said antigenic peptide corresponds to an epitope of the integrase coded for by the *pol* gene of HIV-1.

3. (Amended) A reagent [Reagent] according to Claim 2, [characterized in that] wherein said antigenic peptide corresponds to the sequence KIQNFRVYYRDSRDPLWKGPAKLLWKGEGAVVIQDN (SEQ ID NO:1) (HIV-POL).

4. (Amended) A reagent [Reagent] according to [any one of Claims 1 to 3, characterized in that] Claim 1, wherein the mixotope corresponds to a degeneration of the whole of the selected antigenic peptide.

5. (Amended) A reagent [Reagent] according to [any one of Claims 1 to 4, characterized in that] Claim 1, wherein the antigenic peptide (1) and the mixotope (2) are attached to a solid support[, preferably microtitre plates].

6. (Amended) A reagent [Reagent] according to Claim 5, [characterized in that] wherein said antigenic peptide (1) and said mixotope (2) are attached to said support sequentially.

7. (Amended) A reagent [Reagent] according to [any one of Claims 1 to 6, characterized in that] Claim 1, wherein the ratio of antigenic peptide to mixotope in the mixture is between 1:10 and 1:100.

8. (Amended) An enzyme [Enzyme] immunological method of diagnosing an HIV-1 infection, [characterized in that it] which employs a diagnostic reagent according to [any one of Claims 1 to 7] Claim 1.

9. (Amended) A method [Method] according to Claim 8, [characterized in that it] which comprises:

- bringing a serum to be analysed into contact with a reagent [according to any one of Claims 1 to 7] comprising a mixture consisting of (1) an antigenic peptide coded for by the *pol* gene of HIV-1 and comprising at most 60 amino acids, and (2) a mixture, called a mixotope, of convergent combinatorial peptides derived from said antigenic peptide;
- adding anti-human Ig antibodies coupled with an enzyme; and
- qualitatively and/or quantitatively disclosing the anti-integrase antibodies which may be present in the serum to be analysed by adding the enzyme substrate.

10. (Amended) A method [Method] according to Claim 8, [characterized in that it] which comprises:

- attaching [a reagent according to any one of Claims 1 to 7] to a support [such as a microtitre plate] a reagent comprising a mixture consisting of (1) an antigenic peptide coded for by the *pol* gene of HIV-1 and comprising at most 60 amino acids, and (2) a mixture, called a mixotope, of convergent combinatorial peptides derived from said antigenic peptide;
- adding the serum to be analysed;
- detecting the attachment of the anti-integrase antibodies present in said serum by adding anti-human IgG antibodies coupled with an enzyme; and
- qualitatively and/or quantitatively disclosing said antibodies in a spectrophotometer by adding the enzyme substrate.

11. (Amended) A kit [Kit] for diagnosing an HIV-1 infection, [characterized in that it] which comprises at least one reagent according to [any one of Claims 1 to 7] Claim 1.

DETECTION AND MONITORING OF HIV INFECTIONS

The present invention relates to a reagent for detecting and monitoring viral infections caused by the human immunodeficiency virus (HIV) and to its applications for the detection of human immunodeficiency.

The first attempts to screen for HIV infection, established in 1983 and 1985, used a viral lysate to capture the anti-HIV antibodies. These tests had the major disadvantage of lacking both sensitivity and specificity. Moreover, because a viral lysate was used, it was difficult to reproduce the viral antigens from one batch to the next and it was necessary to prepare virus cultures in large quantities, which was hazardous for the operator.

Knowledge of the complete sequence of HIV-1 (S. Wain-Hobson et al., Cell, 1985, **40**, 9 - 17) opened the way to new approaches to antigen production.

Thus European patents EP 181 150 and EP 387 914 describe the use of genetic engineering for obtaining polypeptides (antigens) for detecting anti-HIV-1 antibodies.

These advances allowed the development of HIV serodiagnosis (detection of the immunoglobulins in the serum). The anti-HIV-1 antibodies produced are thus detected by reaction with one or more antigens which react more or less specifically with said antibodies.

However, the immunoassays which use such reagents generally have a low sensitivity, especially when there is a low affinity between the test antibody and the selected antigen. This is particularly the case with a recent seroconversion and/or with the appearance of a new HIV subtype.

In fact, such an immunoassay must be sensitive, reliable, specific, simple and rapid; however, the sensitivity of these tests depends essentially on the choice of antigen or reagent, which is why numerous researches have been carried out to develop more sensitive and more specific antigens.

To solve the particular problem of lack of sensitivity, some authors have proposed the use of different antigens in combination.

The various approaches which have been used are as follows:

- use of one or more synthetic peptides containing different HIV coding regions (J. Wang et al., PNAS, 1986, **83**, 6159 - 6163); patent application EP 220 273, for example, describes a series of peptides containing 6 to 49 amino

acids and especially a peptide containing the immunodominant epitope of the transmembrane glycoprotein gp41 (peptide 39);

- use of labelled peptide antigens originating from an HIV domain (patent US 5 221 610); and

- use of biotinylated peptides derived from gp41, the V3 loop or gp120: WO 93/18054; EP 307 149.

The work on synthetic peptides (J. Wang et al., PNAS, 1986, **83**, 6159 - 6163 and patent application EP 220 273, for example) demonstrated the value of using synthetic peptides and mixtures thereof, instead of recombinant proteins or the complete virus (viral lysate), for detecting anti-HIV antibodies with a view to improving the quality of the immunoassays.

Tests based on the use of said peptides performed better in terms of sensitivity, specificity and reproducibility, the cost price of the reagents was lower and there was no danger of contamination associated with cultivation of the virus. This work was subsequently followed up by numerous groups (patent application EP 0 278 148, patent application EP 0 214 709).

However, these synthetic peptides and mixtures thereof are still unable to avoid false negatives (insufficient sensitivity).

In order to achieve a sufficient sensitivity to be able to detect recent sero-conversions and the new HIV subtypes, it was proposed (patent application EP 0 857 731) to use the following reagents:

* peptide mixtures obtained from a sequence comprising a variable epitopic domain derived from gp41 or gp120; said peptides have a length of 6 to 50 amino acids, constitute variants of said epitopic domain (sequence homologies of at least 30% with the native epitopic domain or a consensus sequence) and contain the following in selectively chosen positions:

- at least one labelling group, activating group or group for binding to a solid phase, and/or

- one or more amino acids selected from a mixture of amino acids selected from known variants of said epitopic domain, or arbitrarily selected;

* multimeric antigenic compositions of the general formula $P^1\{P^2[P^3(P^4)_t]_s\}_r$, in which P^1, P^2, P^3 and P^4 are peptide mixtures as defined above, r = 1 or 2, s = 0 to 4 and t = 0 to 8; or

* polyhapten compositions of the general formula $(P)_nT(-L)_m$ or $T(-P-L_m)_n$,

in which T is a support, P is the peptides of the peptide mixture as defined above, L is labelling groups or groups for binding to a solid phase, n is an integer between 2 and 100 and m is an integer between 1 and 10.

These different compositions or mixtures generally contain between 2 and 2000 and up to 10^{10} peptides comprising different individual peptide sequences which are as close as possible to a predefined statistical distribution; they can be used as immunological reagents.

However, even though these mixtures afford a better sensitivity, they are incapable in particular of providing an effective solution to the problem of the reliable detection of new virus subtypes and mutated viruses. Now, HIV-1 is subject to frequent mutations; in this context, some of these viruses are poorly detected, if at all, by these tests precisely because of the mutations they carry, the consequence being that subjects contaminated with these viruses are not detected (false negatives).

It is for this reason that the Applicant set out to provide a novel reagent for detecting HIV infections which is capable of being used in enzyme immunoassays, is both specific and sensitive and affords an increase in sensitivity of at least 15 to 30% compared with the reagents of the prior art. Such a reagent meets practical needs better than the reagents of the prior art within the framework of enzyme immunoassays, especially of the ELISA type.

This novel reagent makes it possible in particular to provide an effective solution to the problem of detecting new virus subtypes and mutated viruses.

The present invention relates to a reagent for detecting an infection caused by a human immunodeficiency virus, characterized in that it comprises a mixture consisting of (1) an antigenic fragment or peptide coded for by the *pol* gene of HIV-1 and comprising at most 60 amino acids, preferably between 20 and 40 amino acids, and (2) a mixture (called a mixotope) of convergent combinatorial peptides derived from said antigenic fragment.

In terms of the present invention, a mixture of (1) and (2) is understood as meaning either the association of (1) and (2) in said mixture or the sequential association of (1) and (2) on a solid support.

In terms of the invention, a mixotope is understood as meaning a mixture of all the combinatorial peptides obtained from the selected antigenic fragment by artificial or constructed degeneration; they are preferably obtained in the course of

a single synthesis and represent the peptide antigen and its variability in its function of recognizing an antibody population; different mixotopes can be obtained from the same peptide; the factors involved in the constitution of a mixotope are:

- on the one hand the percentage degeneration of the selected native antigenic peptide (total or partial degeneration); and

- on the other hand the mode of selecting the amino acid substitution of said native antigenic peptide; for each position of the sequence of the chosen native antigenic peptide, the amino acid substitution is selected on the basis of the replacement template established by H.M. GEYSEN et al. (*J. Mol. Recog.*, 1988, 1, 32 - 41) or modified as illustrated in Figure 1, taking the antibody recognition tolerance into account, as a function of the amino acid substitution in the linear epitopes: for a given position, it is preferable to choose the amino acids with the highest percentage "replaceability". However, it is preferable to take into account the conformation of the natural epitopes prior to degeneration.

In terms of the present invention, the mixotope consisting of convergent combinatorial peptides derived from a native antigenic peptide therefore represents an artificial and unnatural degeneration of the native structure by the systematic or partial replacement of each amino acid with another amino acid derived from GEYSEN's replaceability template or from the template shown in Figure 1.

Said mixotope, which can also be called a convertope, differs from the mixotopes described in H. GRAS-MASSE et al. (*Peptide Research,* 1992, 5, 4, 211 - 216), which are divergent peptides obtained by natural degeneration taking into account the natural antigenic variations or antigenic variations frequently observed in the course of evolution, said peptides being intended for vaccination purposes.

Again in terms of the invention, a constructed replaceability template is understood as meaning a template which does not reproduce the natural variations or variations frequently observed during evolution; GEYSEN's replaceability template or the template shown in Figure 1 is particularly suitable.

Surprisingly, the reagents according to the invention make it possible to obtain reliable, reproducible, very sensitive and very specific results insofar as the combinations according to the invention exhibit a synergistic effect in the detection of the antibodies induced by the viruses; in particular, a 15 to 30% increase in

sensitivity is obtained.

In one advantageous embodiment of the reagent according to the invention, said antigenic peptide corresponds to an epitope of the integrase coded for by the *pol* gene of HIV-1 and preferably corresponds to the sequence KIQNFRVYYRDS-RDPLWKGPAKLLWKGEGAVVIQDN (SEQ ID NO:1) (HIV-POL); it has the advantage of possessing only a few natural permutations within the groups M and O and class M subtypes.

According to one advantageous provision of this embodiment, said selected antigenic peptide (HIV-POL) has been rigorously degenerated on the basis of the replacement template established by GEYSEN et al., mentioned above, or modified as illustrated in Figure 1, which affords more than 10^{10} peptides.

Unexpectedly, combining an epitope of the integrase (preferably immuno-dominant fragment) with a mixotope derived from said epitope significantly improves the sensitivity and specificity of the enzyme immunological serodiagnosis of HIV-1; in fact, such a reagent will make it possible to detect antibodies with a low affinity for the native sequence. The combination of native peptide with mixotope, called MIXO(HIV-POL), makes it possible to increase the reactivity of the mixture of antigens towards the antibodies naturally produced against the parent structure (high and low affinity for the latter).

In particular, the combination of HIV-POL with its mixotope, MIXO(HIV-POL), makes it possible to increase the sensitivity of the serodetection by at least 15% while at the same time exhibiting 100% specificity.

The mixture of degenerated peptides artificially produced during one and the same synthesis is combined with the native peptide. Unexpectedly, such a combination makes it possible to increase the reactivity of the mixture of antigens produced against the antibodies naturally induced by the virus.

In another advantageous embodiment of the reagent according to the invention, the antigenic peptide (1) and the mixotope (2) are attached to a solid support, preferably microtitre plates.

According to one advantageous provision of this embodiment, said peptide (1) and said mixotope (2) are attached to said support sequentially.

In another advantageous embodiment of the reagent according to the invention, the ratio of antigenic fragment to mixotope in the mixture is between 1:10 and 1:100.

The present invention further relates to an enzyme immunological method of diagnosing an HIV-1 infection, characterized in that it employs a reagent according to the invention.

In one advantageous mode of carrying out said method, it comprises:

- bringing a serum to be analysed into contact with a reagent as defined above;
- adding anti-human Ig antibodies coupled with an enzyme; and
- qualitatively and/or quantitatively disclosing the anti-integrase antibodies which may be present in the serum to be analysed by adding the enzyme substrate.

In another advantageous mode of carrying out said method, it comprises:

- attaching a reagent according to the invention to a support such as a microtitre plate;
- adding the serum to be analysed;
- detecting the attachment of the anti-integrase antibodies present in said serum by adding anti-human IgG antibodies coupled with an enzyme; and
- qualitatively and/or quantitatively disclosing said antibodies in a spectrophotometer by adding the enzyme substrate.

The present invention further relates to a kit for diagnosing an HIV infection, characterized in that it comprises at least one reagent according to the invention.

Apart from the foregoing provisions, the invention also comprises other provisions which will become apparent from the following description referring to Examples of how to carry out the method forming the subject of the present invention, and to the attached drawings, in which:

- Figure 1 illustrates an amino acid replacement template modified relative to that of H.M. GEYSEN (reference cited above); the mixotope MIXO(HIV-POL), degenerated form, is obtained by systematic replacement of each amino acid with its homologue taken from GEYSEN's replaceability template.
- Figure 2 illustrates the amino acid composition of the peptide HIV-1-POL determined 24 h after total acid hydrolysis (TAH) (black histograms), compared with the theoretical composition calculated on the basis of an equimolecular amount of each amino acid introduced into the degenerated positions (white histograms). The one-letter code is used for the amino acids. B represents Asn or Asp; Z represents Glu and Gln.

- Figure 3 shows the amino acid composition of the mixotope MIXO(HIV-POL) determined 24 h after total acid hydrolysis (TAH) (black histograms), compared with the theoretical composition calculated on the basis of an equimolecular amount of each amino acid introduced into the degenerated positions (white histograms). The one-letter code is used for the amino acids. B represents Asn or Asp; Z represents Glu and Gln.

- Figure 4 illustrates the IgG reactivity of 20 HIV-1-seropositive sera (¦) and 26 HIV-1-seronegative sera (O), characterized by immunofluorescence with the peptide HIV-POL attached to a solid support at a rate of 0.1 µg/well (Figure 4A) or at a rate of 1 µg/well (Figure 4B). The horizontal line represents the threshold value corresponding to the mean obtained with the seronegative sera + 3 standard deviations (SD). These Figures show the number of sera on the abscissa and the absorbance at 492 nm on the ordinate and correspond to an ELISA using the HIV-POL sequence (SEQ ID NO:1).

- Figure 5 shows the effect of the mixotope MIXO(HIV-POL) on the IgG reactivity of the HIV-1-seropositive sera (¦) and the seronegative sera (O) at two different concentrations - 1 µg/well (Figure 5A) or 10 µg/well (Figure 5B) - in an ELISA. The horizontal line represents the threshold value corresponding to the mean of the control sera + 3 SD. These two Figures show the number of sera on the abscissa and the absorbance at 492 nm on the ordinate.

- Figure 6 illustrates the effect of the association of peptide HIV-POL + mixotope MIXO(HIV-POL) on the IgG reactivity of the seropositive sera (¦) and seronegative sera (O) in ELISAs. Each well in the microtitre plate is sequentially coated with 1 µg of peptide HIV-POL and then with 10 µg of mixotope MIXO(HIV-POL) and brought into contact with the sera.

It must be clearly understood, however, that these Examples are given solely in order to illustrate the subject of the invention without in any way implying a limitation.

EXAMPLE 1: Preparation of the reagents according to the invention

a) Synthesis of the peptide:

The native peptide HIV-POL (SEQ ID NO:1) is synthesized in the solid phase using the conventional strategy of the Boc-benzyl (or Fmoc) type in an automated peptide synthesizer (model 430A, Applied Biosystems Inc.). The

protective groups on the side chains are as follows: Asn (Trt), Gln (Trt), Asp (OChx), Glu (OChx), Ser (Bzl), Thr (Bzl), Arg (Tos), Cys (4-MeBzl) and His (Dnp) (cf. R.C. SHEPPARD, *Peptide Synthesis, Comp. Org. Chem.*, 1979, **5**, 321 - 363).

The amino acids are introduced using the HBTU/HOBt activation protocol with systematic double coupling on a Boc-Gln-Pam resin (Applied Biosystems). After thiolysis of the dinitrophenyl group, Dnp, followed by final deprotection and cleavage with hydrofluoric acid, the cleaved deprotected peptide is precipitated and washed with cold diethyl ether, then dissolved in 5% acetic acid and lyophilized.

The peptide is purified to more than 90% on a 100 A Nucleosil C18 preparative RP-HPLC column of 5 mm x 250 mm (Macherey Nagel, Düren, Germany) and said peptide is then characterized.

The homogeneity is confirmed by analytical HPLC on a Vydac C18 column eluted with a solvent system (TFA/acetonitrile/water) in a Shimadzu apparatus.

The purity of the peptide, which is greater than 96%, is determined by analytical reversed-phase HPLC.

The sequence identity of the purified peptide is confirmed by determination of the amino acid composition and by mass spectrometry (MALDI) (calculated: 4258.9 $[M+H]^+$, found: 4260.0).

The amino acid composition (with the exception of tryptophan), controlled by total acid hydrolysis (TAH), is shown in Figure 2. Valine is found to be underrepresented (Figure 2), this being justified by a concatenation of aliphatic amino acids which are difficult to hydrolyse and include two of the three valines (AVVI) of the native peptide HIV-POL.

b) Preparation of the mixotopes:

These are prepared as described in H. GRAS-MASSE et al., cited above.

Briefly, equimolar amounts of protected amino acids are weighed out and used in the coupling reactions.

To compensate for the kinetic differences in the reactivities of the different amino acids, a first coupling is performed with 1 mmol (total amount) of Boc-amino acid (or a mixture of Boc-amino acids). A second coupling, using 2 mmol (total amount), is then performed systematically. After cleavage with hydrofluoric acid, the crude peptide is dissolved in TFA (30 ml) and precipitated by adding said

peptide to a solution of cold diethyl ether (300 ml).

After centrifugation, the precipitate is dissolved in water and lyophilized. After oxidation of the solution in air at neutral pH, the mixotope is purified by gel filtration on a TSK HW 40S column (Merck, Darmstadt, Germany). An aliquot of each purified mixotope is subjected to total acid hydrolysis for 24 hours with a mixture of 6 N HCl and phenol (10:1) in order to determine the amino acid composition.

The replacement of the amino acids of MIXO(HIV-POL) is specified in Figure 1. After purification by gel filtration (TSK HW 40S), a sample of the fraction used in the ELISAs is subjected to TAH, giving the result shown in Figure 3. As for TAH of the native peptide (HIV-POL), valine (V), but also isoleucine (I), are found to be underrepresented after TAH of the MIXO(HIV-POL). The fact that valine has been replaced by isoleucine in the mixotope and forms part of a highly hydrophobic sequence of the mixotope, which is therefore difficult to hydrolyse, provides an explanation for the reduced amounts of Val and Ile in the result of this TAH (Figure 3).

c) Examples of different reagents according to the invention:

The sequences of the peptide HIV-POL and the mixotopes derived therefrom are shown in Figure 1.

These reagents are preferably attached to a solid support (microplate) at a concentration of 0.1 µg/well for the peptide HIV-POL and at a concentration of 10 µg/well for the mixotopes.

EXAMPLE 2: Enzyme immunoassay employing a reagent according to the invention for the serodetection of HIV-1

A. Equipment and methods:

- ELISA:

Wells of microtitre plates (Nunc, Maxisorp, Rocksilde, Denmark) are coated overnight at 4°C either with 0.2 ml of peptide HIV-POL (SEQ ID NO:1), or with a mixotope MIXO(HIV-POL) (0.5 µg/ml in 50 mM $NaHCO_3$, pH 9.6), or sequentially with 0.2 ml of peptide HIV-POL (0.5 µg/ml) and 0.2 ml of mixotope MIXO(HIV-POL) (50 µg/ml).

Each well is then washed with 0.01 M phosphate buffer containing 1.8% NaCl, pH 7.4 (PBS), and the excess binding sites are blocked with albumin

(addition of 0.3 ml of 2% BSA (bovine serum albumin) in PBS at 37°C for 60 minutes).

After 3 washes with 0.3 ml of PBS containing 0.5% Tween 20 (Sigma) (PBS-T), the human test sera are diluted to 1/50 in PBS-T containing 2% bovine serum albumin (BSA) and are incubated in wells containing the reagent according to the invention, as specified above, for 120 minutes at 37°C in a humidified atmosphere.

After 4 washes, peroxidase/anti-human IgG-A-M goat antibody conjugates (Diagnostic Pasteur), diluted to 1/10,000 in PBS-T containing 2% BSA, are incubated for 60 minutes at 37°C.

The conjugated antibody, which binds to the Igs attached to the support, is disclosed for its peroxidase activity, the substrate used being *o*-phenylenediamine dihydrochloride and H_2O_2 in 0.05 M citrate buffer, pH 5.5, for 30 minutes in the dark at room temperature.

The reaction is blocked by adding 4 N H_2SO_4 (50 µl). The absorbance is recorded against a blank at 492 nm (A_{492}) with an automatic multichannel reader (M_R 5000, Dynatech).

The mean of A_{492} + 3 standard deviations (SD) for the seronegative samples is used as the threshold value in the ELISAs.

- Measurement of the avidity of the binding to the antibody:

The specificity of binding of the positive sera to the different mixotopes in the solid phase is evaluated by absorbing the antibodies with the native antigen HIV-POL in solution using the method of B. FRIGUET et al. (J. Immunol. Methods, 1985, 77, 305 - 319).

This method is based on measurement of the free antibody concentration by an indirect ELISA method when the antigen HIV-POL and the antibodies are in equilibrium in solution.

The antigen HIV-POL, at different concentrations (10^{-10} M to 2.10^{-6} M), is first incubated in solution (PBS-T + 2% BSA) with a seropositive serum at constant concentration (1/50) until the equilibrium state is reached.

After incubation for 18 hours at 4°C, 200 µl of each mixture are transferred to the wells of a microtitre plate, previously coated with peptide HIV-POL (0.2 ml, corresponding to 0.5 µg/ml) or a mixotope MIXO(HIV-POL) (50 µg/ml, 0.2 ml) in 50 mM $NaHCO_3$, pH 9.6, and are incubated for 60 min at 20°C.

After washing with PBS-T, the bound immunoglobulins are detected by adding anti-human IgG-A-M goat antibodies coupled with peroxidase.

The conjugated antibody, which binds to the Igs, is disclosed by the peroxidase activity as described above. This method gives the curves of binding displacement A/Ao as a function of log(ao). A precise estimation of the mean avidity of the serum containing the anti-HIV-POL antibodies is given by the equation $Ao/(Ao - A) = 1/\nu = 1 + Kd/ao$, in which ao is the concentration of total soluble antigen, A and Ao are the absorbances at 492 nm with and without blocking antigen, respectively, and ν is the fraction of bound antibody, provided the various conditions explained in FRIGUET et al. are satisfied.

B. Results:

a) Binding of serum antibodies to HIV-POL (HIV-POL ELISA):

The reactivity of the anti-HIV-POL human IgGs towards the peptide HIV-POL is analysed by ELISA on different sera: 20 seropositive sera (confirmed by western blotting) and 26 seronegative sera.

65% of the HIV-1-seropositive sera (13 out of 20 sera), diluted to 1/50, are detected with the antigen HIV-POL used at a coating concentration of 0.1 µg/well (Figure 4A). A higher concentration (1 µg/well) results in the appearance of a false-positive serum, causing the specificity to drop from 100% to 95% (Figure 4B).

b) Binding of serum antibodies to mixotopes (mixotope ELISA):

The mixotopes were tested as antigens in the solid phase at two concentrations: 0.1 µg and 10 µg/well (Figure 5).

The use of MIXO(HIV-POL) by itself in the ELISAs (Figures 5A and 5B) is not satisfactory because the sensitivity of IgG detection does not exceed 50% at the best coating concentration (Figure 5B).

With the mixotope MIXO(HIV-POL), on the other hand, a decrease is observed in the signal produced by the sera of HIV-1-seronegative patients. This property of the mixotope is utilized in the peptide combination test.

c) Binding of serum antibodies to reagent according to the invention (combinations of peptide HIV-POL + mixotope) (HIV-POL + mixotope ELISA):

If the ELISA plates are coated sequentially with the native peptide at a concentration of 1 µg/well, resulting in a decrease in specificity (Figure 4B), and then with its mixotope MIXO(HIV-POL) at its most effective concentration (10

μg/well), it is observed (Figure 6) that this combination does not cause any false-positive sera to appear and, in particular, it makes it possible to increase the detection of HIV-1-positive sera from 65% (native peptide by itself) to 80%. The combination of HIV-POL with its mixotope MIXO(HIV-POL) afforded a 15% increase in the sensitivity of the serodetection while at the same time exhibiting 100% specificity.

As is apparent from the foregoing description, the invention is in no way limited to those modes of execution, embodiments and modes of application which have now been described more explicitly; on the contrary, it encompasses all the variants thereof which may occur to those skilled in the art, without deviating from the framework or the scope of the present invention.

CLAIMS

1. Reagent for detecting an infection caused by a human immunodeficiency virus, characterized in that it comprises a mixture consisting of (1) an antigenic peptide coded for by the *pol* gene of HIV-1 and comprising at most 60 amino acids, preferably between 20 and 40 amino acids, and (2) a mixture, called a mixotope, of convergent combinatorial peptides derived from said antigenic peptide.

2. Reagent according to Claim 1, characterized in that said antigenic peptide corresponds to an epitope of the integrase coded for by the *pol* gene of HIV-1.

3. Reagent according to Claim 2, characterized in that said antigenic peptide corresponds to the sequence KIQNFRVYYRDSRDPLWKGPAKLLWKGEGAV-VIQDN (SEQ ID NO:1) (HIV-POL).

4. Reagent according to any one of Claims 1 to 3, characterized in that the mixotope corresponds to a degeneration of the whole of the selected antigenic peptide.

5. Reagent according to any one of Claims 1 to 4, characterized in that the antigenic peptide (1) and the mixotope (2) are attached to a solid support, preferably microtitre plates.

6. Reagent according to Claim 5, characterized in that said antigenic peptide (1) and said mixotope (2) are attached to said support sequentially.

7. Reagent according to any one of Claims 1 to 6, characterized in that the ratio of antigenic peptide to mixotope in the mixture is between 1:10 and 1:100.

8. Enzyme immunological method of diagnosing an HIV-1 infection, characterized in that it employs a diagnostic reagent according to any one of Claims 1 to 7.

9. Method according to Claim 8, characterized in that it comprises:

- bringing a serum to be analysed into contact with a reagent according to any one of Claims 1 to 7;
- adding anti-human Ig antibodies coupled with an enzyme; and
- qualitatively and/or quantitatively disclosing the anti-integrase antibodies which may be present in the serum to be analysed by adding the enzyme substrate.

10. Method according to Claim 8, characterized in that it comprises:

- attaching a reagent according to any one of Claims 1 to 7 to a support such as a microtitre plate;

- adding the serum to be analysed;

- detecting the attachment of the anti-integrase antibodies present in said serum by adding anti-human IgG antibodies coupled with an enzyme; and

- qualitatively and/or quantitatively disclosing said antibodies in a spectrophotometer by adding the enzyme substrate.

11. Kit for diagnosing an HIV-1 infection, characterized in that it comprises at least one reagent according to any one of Claims 1 to 7.

HIV-POL	K	I	Q	N	F	R	V	Y	Y	R	D	S	R	D	P	L	W	K	G	P	A	K	L	L	W	K	G	E	G	A	V	V	I	Q	D	N
MIXO(HIV-POL)	K	I	Q	N	F	R	V	Y	Y	R	D	S	R	D	P	L	W	K	G	P	A	K	L	L	W	K	G	E	G	A	V	V	I	Q	D	N
	R	V	N	Q	L	K	I	F	F	K	E	H	K	E	Q	I	F	R			G	R	I	I	F	R		D		G	I	I	V	N	E	Q

1/5

FIGURE 1

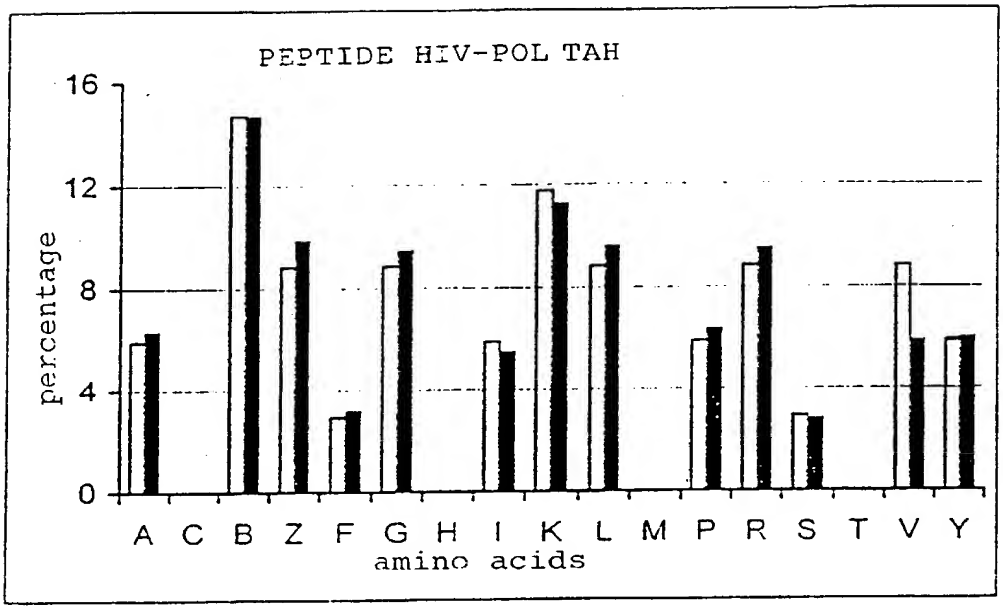
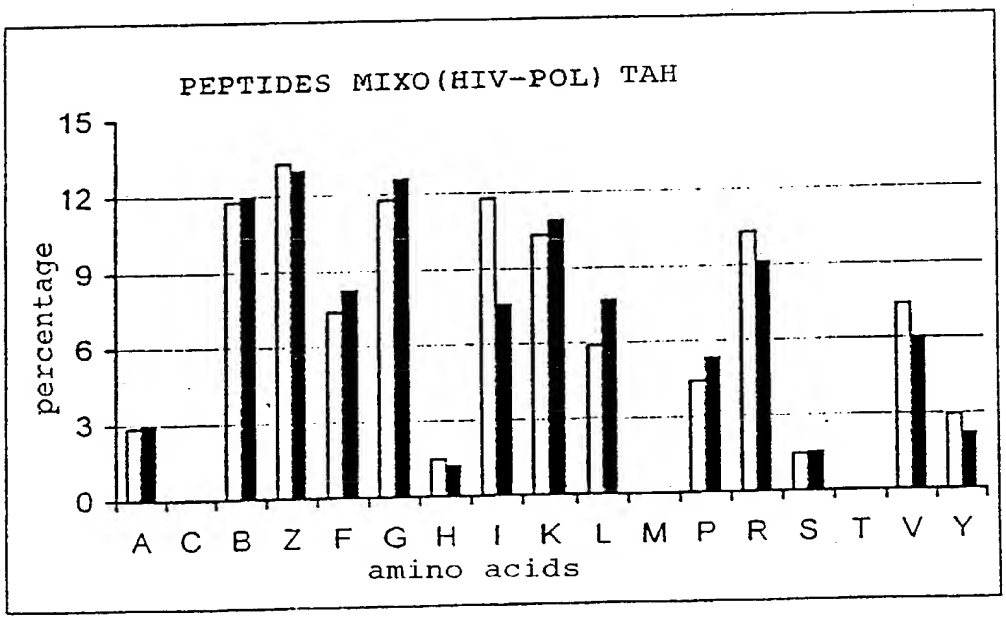


FIGURE 2



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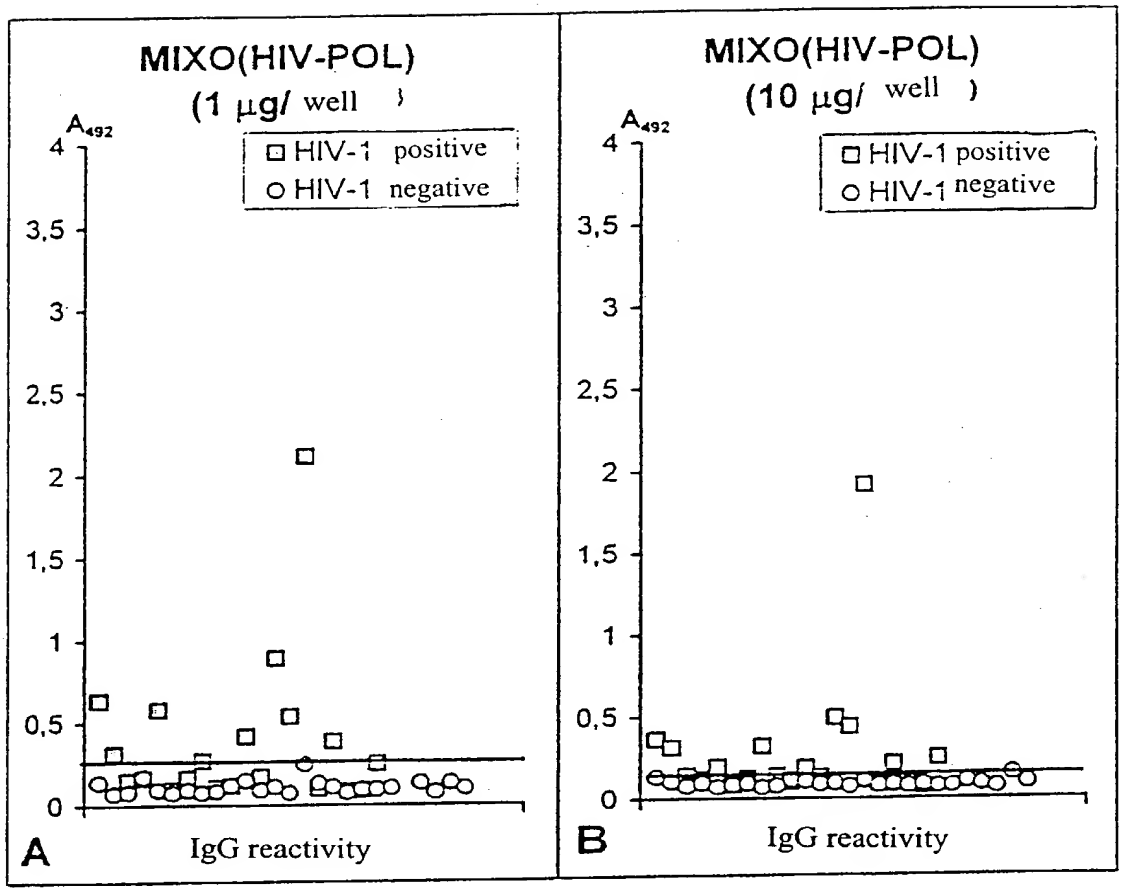
MIXO(HIV-POL)
(1 µg/ well)
A492
HIV-1 positive
HIV-1 negative
4
3,5
3
2,5
2
1,5
1
0,5
0
A
IgG reactivity
MIXO(HIV-POL)
(10 µg/ well)
A492
HIV-1 positive
HIV-1 negative
4
3,5
3
2,5
2
1,5
1
0,5
0
B
IgG reactivity

FIGURE 5

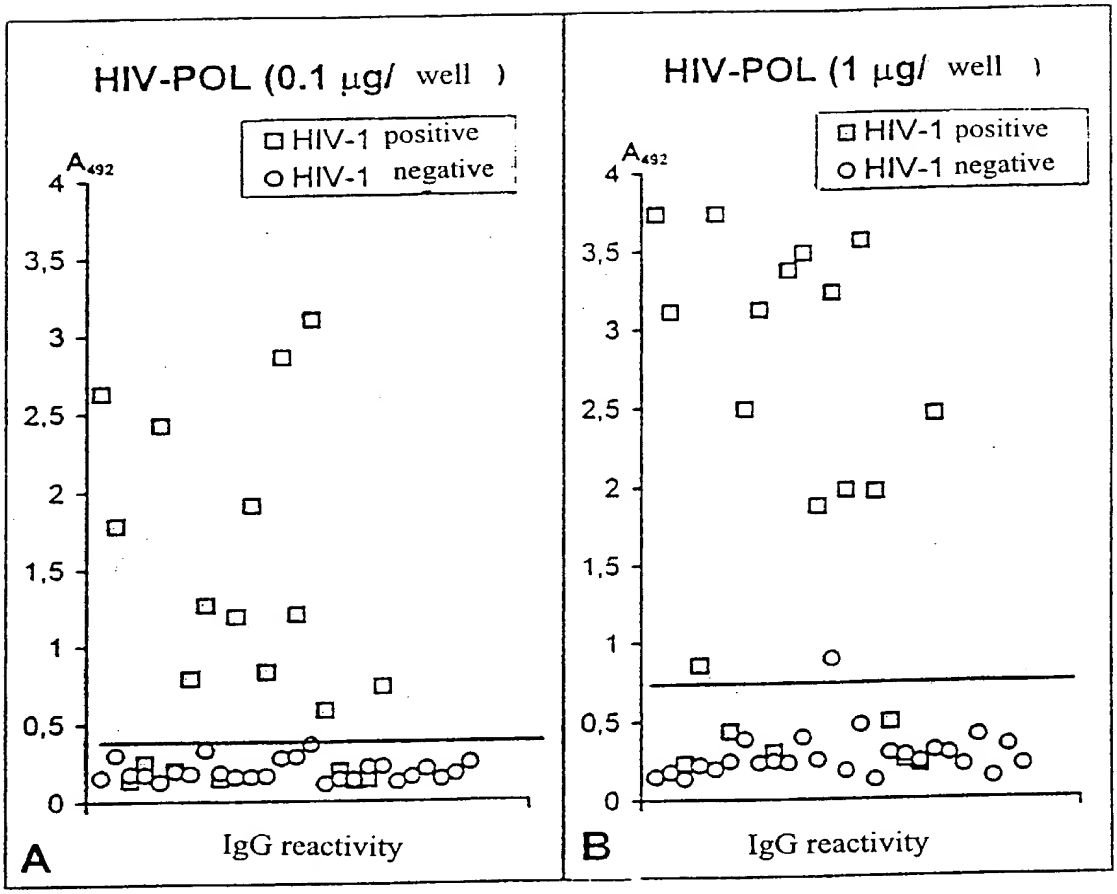
HIV-POL (0.1 µg/ well)
HIV-1 positive
HIV-1 negative
A492
4
3,5
3
2,5
2
1,5
1
0,5
0
A
IgG reactivity
HIV-POL (1 µg/ well)
HIV-1 positive
HIV-1 negative
A492
4
3,5
3
2,5
2
1,5
1
0,5
0
B
IgG reactivity

FIGURE 4

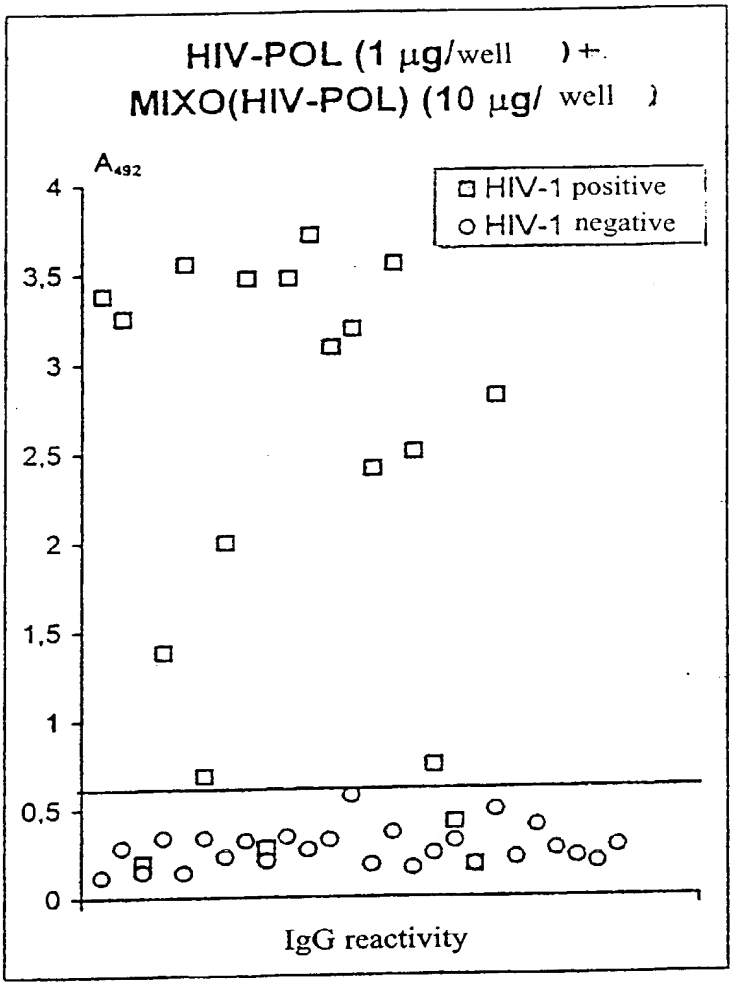
HIV-POL (1 µg/well)+
MIXO(HIV-POL) (10 µg/ well)
A492
HIV-1 positive
HIV-1 negative
4
3,5
3
2,5
2
1,5
1
0,5
0
IgG reactivity

FIGURE 6

Declaration and Power of Attorney for Patent Application
Déclaration et Pouvoirs pour Demande de Brevet
French Language Declaration

En tant l'inventeur nommé ci-après, je déclare par le présent acte que :

Mon domicile, mon adresse postale et ma nationalité sont ceux figurant ci-dessous à côté de mon nom.

Je crois être le premier inventeur original et unique (si un seul nom est mentionné ci-dessous), ou l'un des premiers co-inventeurs originaux (si plusieurs noms sont mentionnés ci-dessous) de l'objet revendiqué, pour lequel une demande de brevet a été déposée concernant l'invention intitulée

et dont la description est fournie ci-joint à moins

☐ ci-joint

☐ a été déposée le

sous le numéro de demande des Etats-Unis ou le numéro de demande international PCT

et modifiée le

(le cas échéant).

Je déclare par le présent acte avoir passé en revue et compris le contenu de la description ci-dessus, revendications comprises, telles que modifiées par toute modification dont il aura été fait références ci-dessus.

Je reconnais devoir divulguer toute information pertinente à la brevetabilité, comme défini dans le Titre 37, § 1.56 du Code fédéral des réglementations.

As a below named inventor, I hereby declare that :

My residence, post office address and citizenship are as stated next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed an for which a patent is sought on the invention entitled

Detecting and monitoring HIV viral infections

the specification of which :

☐ is attached hereto.

☐ was filed on

as United States Application Number or PCT International Application Number. **PCT/FR99/03311 filed on December 29, 1999**

and was amended on

(if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations,§ 1.56.

French Language Declaration

Je revendique par le présent acte avoir la priorité étrangère, en vertu du Titre 35, § 119(a)-(d) ou § 365(b) du Code des Etats-Unis, sur toute demande étrangère de brevet ou certificat d'inventeur ou, en vertu du Titre 35, § 365(a) du même Code, sur toute demande internationale PCT désignant au moins un pays autre que les Etats-Unis et figurant ci-dessous et, en cochant la case, j'ai aussi indiqué ci-dessous toute demande étrangère de brevet, tout certificat d'inventeur ou toute demande internationale PCT ayant date de dépôt précédant celle de la demande à propos de laquelle une priorité est revendiquée.

I hereby claim foreign priority under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below, and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign application(s)
Demande(s) de brevet antérieure(s) dans un autre pays.

(Number) (Numéro)	(Country) (Pays)	(Day/Month/Year Filed) (Jour/Mois/Anné de dépôt)	Priority claimed Droit de priorité revendiqué: Yes Oui	No Non
98/16727	**France**	**December 31, 1998**	☒	☐
(Number) (Numéro)	(Country) (Pays)	(Day/Month/Year Filed) (Jour/Mois/Anné de dépôt)	☐	☐

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35, § 119(e) du Code des Etats-Unis, de toute demande de brevet provisoire effectuée aux Etats-Unis et figurant ci-dessous.

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

(Application No.) (N° de demande)	(Filing Date) (Date de dépôt)

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35, § 120 du Code des Etats-Unis, de toute demande de brevet effectuée aux Etats-Unis, ou en vertu du Titre 35, § 365© du même Code, de toute demande internationale PCT désignant les Etats-Unis et figurant ci-dessous et, dans la mesure où l'objet de chacune des revendications de cette demande de brevet n'est pas divulgué dans la demande antérieure américaine ou internationale PCT, en vertu des dispositions du premier paragraphe du Titre 35, § 112 du code des Etats-Unis, je reconnais devoir divulguer toute information pertinente à la brevetabilité, comme défini dans le Titre 37, § 1.56 du Code fédéral des réglementations, dont j'ai pu disposer entre la date de dépôt de la demande antérieure et la date de dépôt de la demande nationale ou internationale PCT de la présente demande :

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

(Application No.) (N° de demande)	(Filing Date) (Date de dépôt)	(Status) (patented, pending,abandoned) (Statut) (breveté, en cours d'examen, abandonné)
(Application No.) (N° de demande)	(Filing Date) (Date de dépôt)	(Status) (patented, pending,abandoned) (Statut) (breveté, en cours d'examen, abandonné)

Je déclare que par le présent acte que toute déclaration ci-incluse est, à ma connaissance, véridique et que toute déclaration formulée à partir de renseignements ou de suppositions est tenue pour véridique ;et de plus, que toutes ces déclarations ont été formulées en sachant que toute fausse déclaration volontaire ou son équivalent est passible d'une amende ou d'une incarcération, ou des deux, en vertu de la section 1001 du Titre 18 du Code de Etats-Unis, et que de telles déclarations volontairement fausses risquent de compromettre la validité de la demande de brevet ou du brevet délivré à partir de celle-ci.

I hebery declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

French Language Declaration

POUVOIRS : En tant que l'inventeur cité, je désigne par la présente l'(les) avocats(s) et/ou agent(s) suivant(s) pour qu'ils poursuive(nt) la procédure de cette demande de brevet et traite(nt) toute affaire s'y rapportant avec l'Office des brevets et des marquees : (mentionner le nom et le numéro d'enregistrement).

POWER OF ATTORNEY : As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to persecute this application and transact all bussiness in the Patent and Trademark Office connected therewith : (list name and registration number)

All practitioners associated with
CUSTOMER NUMBER 000826

RAYMOND O. LINKER, JR. Registration No. 26,419

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(nom et numéro de téléphone)

Direct Telephone calls to : (name and telephone number)

(704) 444-1000

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Signature de l'inventeur Date 22/06/01	Second inventor's signature Date
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Nationalité Française	Citizenship
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GRAS-MASSE Helene 22/06/01

(Fournir les mêmes renseignements et la signature de tout co-inventeur supplémentaire.)

(Supply similar information and signature for third and subsequent joint inventors.)

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Nationalité - française

French Language Declaration

Nom complete du troisième co-inventeur, le cas échéant **GRAS-MASSE Hélène**	Full name of third joint inventor, if any
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Nationalité Française	Citizenship
Adresse Postale 321, Rue de la Rosière 59710 MERIGNIES (FR)	Post Office Address
Nom complete du quatrième co-inventeur, le cas echeant	Full name of fourth joint inventor, if any
Signature de l'inventeur Date	Fourth inventor's signature Date
Domicile	Residence
Nationalité	Citizenship
Adresse Postale	Post Office Address
Nom complete du cinquième co-inventeur, le cas echeant	Full name of fifth joint inventor, if any
Signature de l'inventeur Date	Fifth inventor's signature Date
Domicile	Residence
Nationalité	Citizenship
Adresse Postale	Post Office Address
Nom complete du sixième co-inventeur, le cas echeant	Full name of sixth joint inventor, if any
Signature de l'inventeur Date	Sixth inventor's signature Date
Domicile	Residence
Nationalité	Citizenship
Adresse Postale	Post Office Address

(Fournir les mêmes renseignements et la signature de tout co-inventeur supplémentaire.)

Supply similar information and signature for third and subsequent joint inventors.)

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Institut Pasteur de Lille
Centre National de la Recherche Scientifique-CNRS

<120> Detection and Monitoring of HIV Viral Infections

<130> 33339/235735

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<151> 1999-12-29

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<400> 1

Lys	Ile	Gln	Asn	Phe	Arg	Val	Tyr	Tyr	Arg	Asp	Ser	Arg	Asp	Pro	Leu
1				5				10						15	
Trp	Lys	Gly	Pro	Ala	Lys	Leu	Leu	Trp	Lys	Gly	Glu	Gly	Ala	Val	Val
			20					25					30		
Ile	Gln	Asp	Asn												
			35												